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Genotype identity determines productivity and CO_2 efflux across a genotype-species gradient of ectomycorrhizal fungi

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ABSTRACT

Ectomycorrhizal (EM) fungal communities are taxonomically diverse, and independent manipulation of both intra- and interspecific diversity has previously been shown to positively influence the productivity and activity of EM fungi. Here, we combine manipulations of intra- and interspecific richness and test the effects of a genotype-species gradient on the biomass production and respiration of EM fungi *in vitro*. Genotype identity had the most pronounced effect on fungal productivity, and in some cases variation within species was greater than between species. We found small negative effects of both species and genotype richness on biomass production, CO_2 efflux and the final nitrogen (N) content of the fungal communities corresponding to mixed negative selection and complementarity effects. Our study highlights the degree of variability between individual EM fungi at the genotype level, and consequently emphasises the importance of individual genotypes for playing key roles in shaping belowground community functioning.

Introduction

enhance community productivity (Reusch *et al.* 2005;

tioning (Naeem *et al.* 1994; Tilman 1996; Naeem & Li 1997; Hector *et al.* 1999), and it has been hypothesised that this may be driven by selection (dominance by a particular species), complementarity effects (facilitative interaction/niche differentiation) or a combination of both effects (Loreau & Hector 2001). A small number of studies have applied species diversity theory to diversity at the level of the genotype, and have found that genotypic diversity can also

there is limited knowledge of the relationship between inter- and intraspecific diversity and how these two fundamental levels of biodiversity interact to influence ecosystem functioning, particularly in ecosystems where genetic diversity can be high, such as within communities of soil fungi.

Both positive (Morishima & Oka 1979; Vellend 2003, 2004; He *et al.* 2008; He & Lamont 2010) and negative (Karlin *et al.* 1984; Fridley *et al.* 2007) correlations have been reported between

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species richness and genotype richness, and niche differentiation and facilitation between the two diversity levels are thought to play an important role in shaping the structure of communities (Vellend 2008). Yet despite their clear potential to interact, many biodiversity-ecosystem functioning studies have dealt with inter- and intraspecific diversity independently. However, Crutsinger *et al.* (2009) studied the effects of intraspecific variation within and among *Solidago* species on decomposition and found that the nutrient concentration of leaf litter varied among individual genotypes of *Solidago altissima*, leading to a 50 % difference in decomposition rates. The effects of genotype identity in mixed litter bags were much stronger than those of genotype diversity, and variation among different *Solidago* species was more than twice that occurring between *S. altissima* genotypes. In terms of ecological importance they concluded that species identity is most important, followed by genotype identity and lastly genotype diversity.

It is uncertain whether the effects of biodiversity seen in plants and animals can be applied to fungi because of their unique morphology, physiology and diversity (Prosser *et al.* 2007). Moreover, the application of biodiversity theory to fungi remains sparsely studied compared to plants and animals, although recently separate manipulations of both inter- and intraspecific ectomycorrhizal (EM) fungal diversity have been shown to influence fungal biomass production and CO_2 efflux (Wilkinson *et al.* 2010, 2012). These studies also highlight the suitability of fungi as test organisms for demonstrating how biodiversity and ecosystem functioning in microbial systems contributes to wider ecological theory. EM communities are well known for being species rich despite the low diversity of their host plant communities (Erland & Taylor 2002). EM communities can also be genetically diverse; nine genotypes of *Tricholoma matsutake* were found within a 100 m^2 plot (Lian *et al.* 2006) and a typical m^2 of forest floor contained ~9 genotypes of *Hebeloma cylindrosporum* (Gryta *et al.* 1997; Guidot *et al.* 2005). Depending on forest age and species, estimates of genet population densities have been found to range from 30 to 5 000 genets ha^{-1} (Dahlberg & Stenlid 1994, 1995). However, several key questions remain: to what extent do physiological and functional attributes vary between genotypes and species of EM fungi, and how does intra- and interspecific diversity in EM communities interact to shape key processes, such as C cycling and microbial productivity? In this study, we address these questions in an *in vitro* setting by manipulating both intra- and interspecific diversity of EM fungi and measuring biomass responses, CO_2 efflux from mycelium, and fungal C and N contents in pure culture. Our overarching hypotheses are: (1) variation between fungal traits (biomass production, CO_2 efflux and C/N content) will be greatest at the species level, as previously found in plant communities (Crutsinger *et al.* 2009); and (2) increases in both genotype and species richness will result in positive changes in EM productivity and respiration.

Materials and methods

Microcosms

A gradient of genotypic richness was created using four different strains of the EM fungal species obtained from independent sporocarps of *Amanita rubescens*, *Piloderma fallax*, *Suillus bovinus* and *Paxillus obscurosporus* (see Table 1). Twenty-

Table 1 – Isolate identification codes and combinations of ectomycorrhizal species and genotypes used in the experiment

Microcosm treatment	Species richness	Genotype richness	Combinations	Isolate identification code
A	1	1	*Amanita rubescens* Genotype 1	AT *A. rubescens* N0113
B	1	1	*A. rubescens* G2	AT *A. rubescens* CN0106
C	1	1	*A. rubescens* G3	AT *A. rubescens* AT2008002
D	1	1	*A. rubescens* G4	DR *A. rubescens* Sheff1
E	1	1	*Piloderma fallax* G1	AT *P. fallax* S421
F	1	1	*P. fallax* G2	AT *P. fallax* S47
G	1	1	*P. fallax* G3	AT *P. fallax* S326
H	1	1	*P. fallax* G4	AT *P. fallax* S57
I	1	1	*Suillus variegatus* G1	AT Cullardoch *S. Variegatus* (1)
J	1	1	*S. variegatus* G2	AT Cullardoch *S. variegatus* (3)
K	1	1	*S. variegatus* G3	AT *S. variegatus* UP597
L	1	1	*S. variegatus* G4	AT *S. variegatus* UP598
M	1	1	*Paxillus involutus* G1	DJPax1
N	1	1	*P. involutus* G2	DJPax2
O	1	1	*P. involutus* G3	DJPax5
P	1	1	*P. involutus* G4	DJPax12
ABCD	1	4	*A. rubescens* G1 + 2 + 3 + 4	
EFGH	1	4	*P. fallax* G1 + 2 + 3 + 4	
IJKL	1	4	*S. variegatus* G1 + 2 + 3 + 4	
MNOP	1	4	*P. involutus* G1 + 2 + 3 + 4	
BEJO	4	4	Least productive genotype of each species: *A. muscaria* G2 + *P. fallax* G1 + *S. variegatus* G2 + *P. involutus* G3	
AFKP	4	4	Most productive genotype of each species: *A. muscaria* G1 + *P. fallax* G2 + *S. variegatus* G3 + *P. involutus* G4	
ALL	4	16	All species and genotypes	

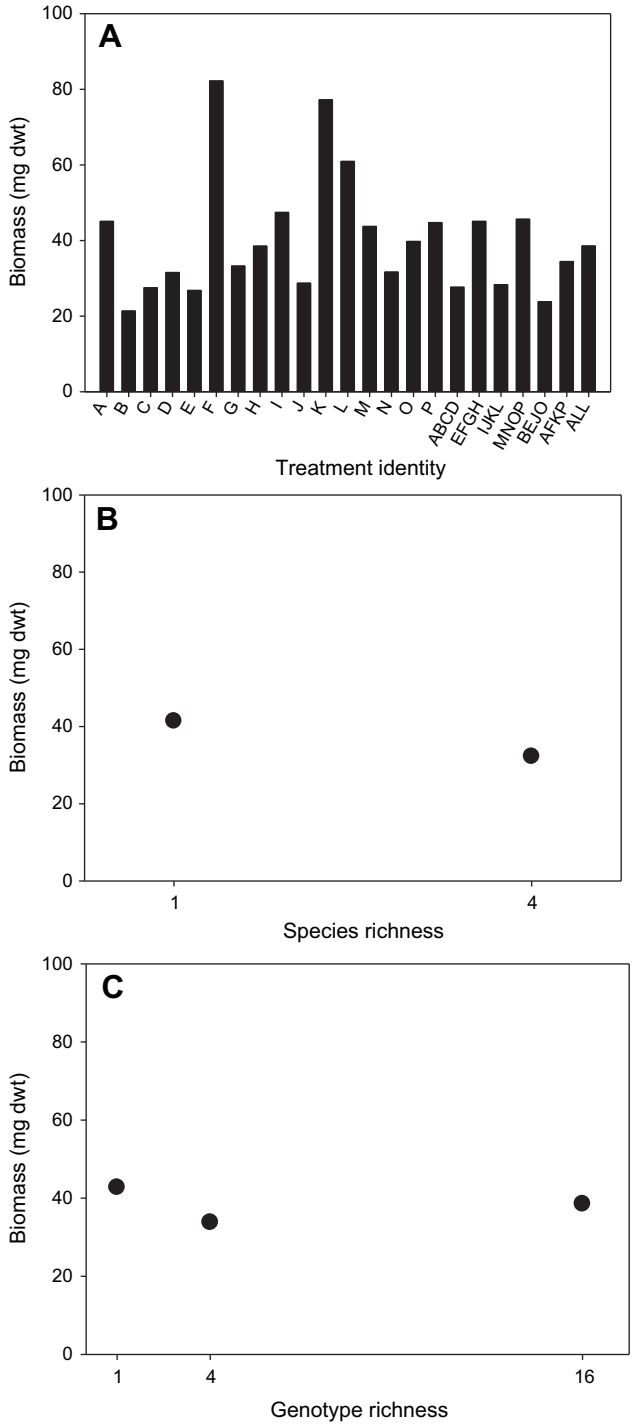

Fig 1 – The effect of (A) treatment identity, (B) species richness and (C) genotype richness on the biomass (mg dwt) of species and genotypes of EM fungi. Treatments A–P are monocultures of all four genotypes of four different species, treatments ABCD-MNOP are mixtures of four genotypes of each species, treatments BEJO and AFKP are mixtures of the "worst-performing" and "best-performing" genotypes of each of the four species and treatment ALL contains all 16 EM fungi. For each graph the bars (A) and circles (B and C) represent predicted values from the optimal regression model. In (A), treatment identity had a significant influence on fungal biomass (L-ratio = 107.85, d.f. = 24, $p < 0.001$). In (B), species richness lead to a significant decrease in fungal biomass (L-ratio = 7.43, d.f. = 3, $p = 0.006$) as did (C) genotype richness (L-ratio = 7.80, d.f. = 4, $p = 0.020$).

three unique treatments were created of which 16 were single genotype monocultures (treatments A–P), four were mixtures of four genotypes of each species (treatments ABCD-MNOP; all mixtures apart from the 16 genotype combination were selected at random without replacement), two were mixtures of all four species (treatments 21 and 22), and one comprised all genotypes (treatment ALL). The two mixtures of all four species consisted of the least productive (produced the lowest amounts of biomass) genotypes of each species (treatment BEJO) and the most productive genotypes of each species (treatment AFKP). This was to give an indication of the likely productivity range that might occur between mixtures of the four species, and the genotypes were chosen after monitoring biomass production in monoculture during the study period. Hence the study period for treatments BEJO and AFKP commenced upon completion of the main study, although experimental conditions remained constant throughout.

Inoculum plugs (3 mm diameter removed from the growing margins of colonies) were transferred to individual, gas-tight 500 ml glass Kilner jars containing 50 ml pH 5.5 sterile modified Melin Norkrans (MMN; Marx 1969) growth media covered with sterile cellophane. Sixteen fungal plugs were used in each treatment arranged in a regular grid, so that each microcosm jar had equal amounts of inoculum at the start of the experiment. There were six replicates for each treatment plus six replicate control (growth media supporting no fungi) treatments that accounted for CO_2 efflux through abiotic pathways (total number of microcosms = 144). Each microcosm contained a vial of 5 ml 1 M NaOH to trap evolved CO_2.The microcosms were kept in the dark at 27 °C. The NaOH samples were removed approximately every 2 d for 18 d and the total amount of CO_2 produced during the experiments was determined by back-titration using a digital burette. After 18 d, when fungal biomass had completely covered the surface of the growth media and CO_2 efflux had declined substantially, the total fungal tissue in each microcosm was scraped from the cellophane surface, dried, weighed and corrected for the weight of the initial inoculum. The dry fungal material from each treatment was analysed for percentage N and C by flash combustion of fungi in an oxygen enriched furnace (1 700–1 800 °C) followed by reduction and separation of the gaseous components by gas chromatography using a thermal conductivity detector (TCD). This was carried out using an NCS analyser (Fisons Instruments, NA 1500 Series). C:N ratios and percentages of N and C were determined in all samples.

Statistical analysis

Regression analyses (SPSS statistics ver. 17.0) were undertaken to determine whether the final tissue C, N and C:N ratios of fungal matter could explain the variation in biomass and CO_2 efflux.

To assess the importance of identity and inter- and intraspecific diversity to EM biomass production and CO_2 efflux,

a generalized least squares (GLS) statistical mixed modelling approach was used (Bulling et al. 2008) which accounts for the unequal variance imposed by the experimental design using suitable variance-covariate functions. Separate models were created where there was co-linearity between treatment identity and diversity variables. The fixed structure of the model was established by applying backward selection using the likelihood ratio test obtained by Maximum Likelihood (ML). The numerical output of the minimal adequate model was obtained using REML estimation (West et al. 2007). These analyses were all performed using the 'nlme' package (ver. 3.1) in the 'R' statistical and programming environment (Pinheiro et al. 2006). The statistical tests used cannot be applied directly to mean values with standard errors but instead relate to model predictions; these are therefore what we present in the main paper. However, boxplots showing the spread of the raw material are also presented in supplementary material (Figs S2–S5). To determine if species and genotypic combinations had positive effects on parameters that were significantly affected by richness (biomass, CO_2 efflux and % N) we compared mixed combinations relative to the best performing monocultures (transgressive overyielding (D_{max}); Trenbath 1974; Loreau 1998). $D_{max} > 0$ if a combination mixture produces more biomass, CO_2 or N than the corresponding monocultures.

Results

Species richness, genotype richness and treatment identity effects on biomass production

Genotypes of all of the species used grew from the inoculum plugs at the beginning of the study. However, due to the degree of intermingling in some communities it was not possible to determine for certain whether all the genotypes survived until the end of the study. Treatment identification (see Table 1) played a significant role in biomass production (Fig 1A, Table 2), with biomass of individual genotypes in monoculture (treatments A–P; Table 1) varying greatly, from 21.33 mg dwt (treatment B, *A. rubescens*) to 82.17 mg dwt (treatment F, *P. fallax*). Interestingly, the large variation in biomass production did not exclusively occur between the four species groups, but rather at the level of the individual genotype, with differences in biomass production of over 55 mg dwt in the case of *P. fallax* genotypes (treatments E–H).

Variation in biomass production decreased in the combination treatments (Fig 1A; treatments ABCD-ALL), with biomass ranging from approximately 23–46 mg dwt. The performance of the mixed genotype combinations (treatments ABCD-MNOP) was not necessarily representative of the performance of the individual genotypes in monoculture. For example, despite the large biomass production of three of the *Suillus variegatus* genotypes in monoculture (treatments I, J and K), when in combination (treatment IJKL) the *Suillus* species produced significantly less biomass than the three aforementioned genotypes ($t = -19.13$, $p = 0.0007$; $t = -48.90$, $p < 0.001$ and $t = -32.61$, $p < 0.001$ respectively), as well as treatment EFGH, the *Piloderma* genotype combination ($t = -16.75$, $p = 0.021$), and treatment MNOP, the *Paxillus* genotypes ($t = -17.33$, $p = 0.003$). Likewise, treatment AFKP, the mixed species treatment containing the large biomass producing genotypes in monoculture, produced less biomass compared to all of its component species in monoculture, and significantly less biomass than treatment F ($t = -47.76$, $p = 0.0010$) and treatment K ($t = -42.78$, $p < 0.001$). Biomass production decreased significantly between monocultures and combinations of up to four species ($t = -9.15$, $p = 0.0057$; Fig 1B, Table 2), and four genotypes ($t = -8.99$, $p = 0.0056$, Fig 1C). However, there were no significant differences between the 16 genotype combination and the monocultures, or the four genotype combinations.

Table 2 – Summary of significant terms found in the linear regression models with a generalized least squares extension, treating biomass, CO_2 efflux, % C, % N and C:N ratio of fungal material as dependent variables, and time, species richness (SR), genotypic richness (GR) and treatment identity (TID) as fixed explanatory variables

Dependent variable	Significant terms	L-ratio	d.f.	*p*
Biomass models				
Model 1	SR	7.43	3	0.006
Model 2	GR	7.80	4	0.020
Model 3	TID	107.85	24	<0.001
CO_2 efflux models				
Model 4	Time	140.45	16	<0.001
	SR	39.26	21	<0.001
	Time × SR	27.22	22	<0.001
Model 5	Time	136.82	24	<0.001
	GR	47.50	28	<0.001
	Time × GR	24.82	30	0.020
Model 6	SR	15.80	3	<0.001
Model 7	GR	19.75	4	<0.001
Model 8	TID	96.55	24	<0.001
Model 9	SR*	–	–	–
Model 10	GR	0.56	2	0.851
Model 11	TID	61.41	24	<0.001
% C models				
Model 12	SR	0.468	3	0.494
Model 13	GR	4.055	4	0.132
Model 14	TID	70.987	24	<0.001
% N models				
Model 15	SR	6.616	3	0.010
Model 16	GR	5.015	4	0.082
Model 17	TID	142.309	24	<0.001
C:N ratio of fungi material				
Model 18	SR	1.668	3	0.197
Model 19	GR	2.904	4	0.234
Model 20	TID	119.35	24	<0.001
Transgressive overyielding (D_{max})				
Model 21	GR	3.39	3	0.066
Model 22	GR[a]	–	–	–
Model 23	GR	0.68	3	0.408
Model 24	GR[a]	–	–	–

a Denotes intercept only model.

Time, species richness, genotype richness and treatment identity effects on CO_2 efflux

Time had the largest effect on the CO_2 efflux of the EM communities (Fig 2, Table 2; GR) followed by richness, with the

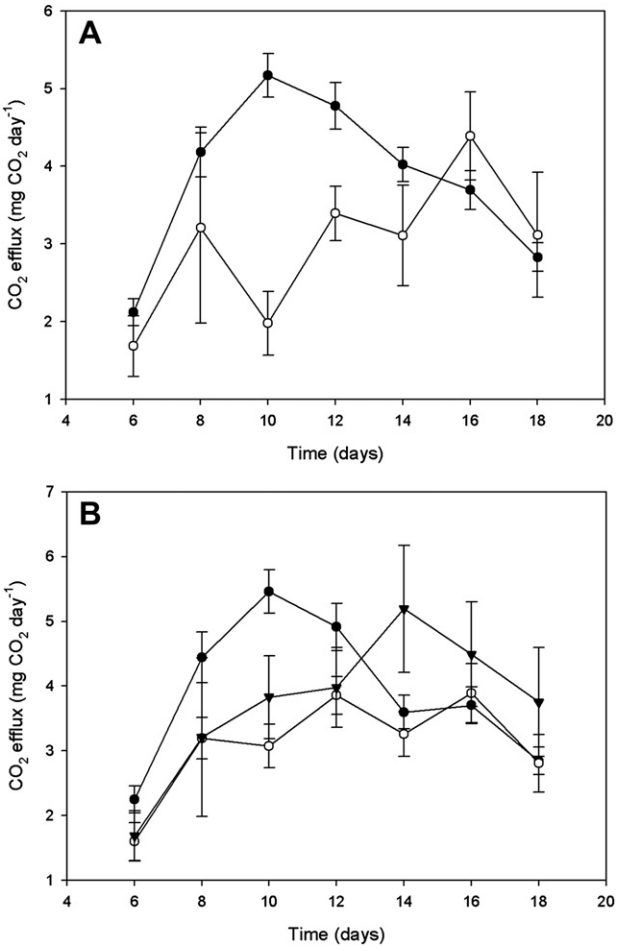

Fig 2 – The effect of (A) genotype richness and (B) species richness of the EM communities on CO_2 efflux (mg CO_2 d^{-1}) across 18 d. In (A), the two lines represent the two SR levels; in each case, the mean is indicated by a symbol (SR1 is represented by a black circle and SR4 by a clear circle) and the error bars represent the spread. A significant difference between mean values is indicated when error bars between time points do not overlap. Both time and SR, and their interaction, had significant influences on the CO_2 production rate of the soil (L-ratio = 140.45, d.f. = 16, $p < 0.001$; L-ratio = 39.26, d.f. = 21, $p < 0.001$; and L-ratio = 27.22, d.f. = 22, $p < 0.001$ respectively). In (B), the three lines represent the three GR levels; in each case, the mean is indicated by a symbol (GR1 is represented by a black circle, GR4 by a clear circle and SR16 by a black triangle) and the error bars represent the spread. As in (A), both time and GR, and their interaction, had significant influences on the CO_2 production rate of the soil (L-ratio = 136.18, d.f. = 24, $p < 0.001$; L-ratio = 47.50, d.f. = 28, $p < 0.001$ and; L-ratio = 24.82, d.f. = 30, $p = 0.020$ respectively).

time interacting significantly with richness. Time played an important role in determining when communities of different richness produced the greatest flux of CO_2. Therefore, further statistical analyses were carried out at two different time periods (subsequently referred to as $t = 10$ and $t = 16$) to see how effects of richness changed between peaks in CO_2 efflux found nearer the start of the study (e.g. monocultures) and those seen towards the end (higher richness treatments).

CO_2 efflux at $t = 10$. The difference in CO_2 efflux from individual genotypes in monoculture (treatments A–P, Fig 3A) was large, ranging from 2.51 mg CO_2 d^{-1} (treatment E, *S. variegatus*) up to 9.04 mg CO_2 d^{-1} (treatment K, *P. fallax*) at $t = 10$. In accordance with results for biomass production there was also a large degree of variation within genotypes of the same species, such as the *P. fallax* genotypes (treatments E–H). However, variation between mixed treatments was much lower than the individual genotypes, ranging from 2.90 to 5.37 mg CO_2 d^{-1}. Treatments I–L, the *Suillus* genotypes, produced significantly more CO_2 than almost all of the other individuals in monoculture, and in fact all of the mixed treatments, with CO_2 efflux rates all above 8 mg CO_2 d^{-1}. However in treatment IJKL, where all of these species were combined, CO_2 efflux was significantly less than all of the component genotypes (I: $t = 4.51$, $p < 0.001$; J: $t = 4.52$, $p < 0.001$; K: $t = 3.33$, $p = 0.001$; and L: $t = 4.80$, $p < 0.001$), and also less than treatment EFGH (the *Piloderma* combination), although this was not significant. Also, despite comprising the greatest biomass producing genotype of each species, treatment AFKP produced lower quantities of CO_2 than treatment BEJO (the worst performing genotypes of each species), although this was not significant. Treatments that produced high levels of biomass were not necessarily high CO_2 producers. For example, treatment F (*P. fallax*) produced significantly more biomass than all other *Piloderma* genotypes (i.e. treatments E: $t = -4.07$, $p < 0.001$; G: $t = -3.43$, $p = 0.001$; and H: $t = -3.19$, $p = 0.002$) as well as two *Suillus* genotype treatments (I: $t = -2.42$, $p = 0.017$; and J: $t = -3.93$, $p < 0.001$). In contrast, in terms of respiration it produced less CO_2 than treatment H (*Piloderma* genotype) and all of the *Suillus* monoculture treatments.

The flux of CO_2 at $t = 10$ decreased significantly with both species richness (L-ratio = 15.80, $p < 0.001$; Fig 3B, Table 2) and genotype richness (L-ratio = 19.75, $p < 0.001$; Fig 3C). The differences in CO_2 efflux between the monocultures and four species/genotype combinations were much larger than those seen in biomass production, with increasing species richness leading to a 40 % decline in respiration in four species combinations ($t = -1.93$, $p < 0.001$; Fig 3B), and genotype richness causing a 30 % decrease in CO_2 efflux ($t = -1.26$, $p = 0.003$; Fig 3C). There was no significant change in respiration between the four species/genotype treatments and the 16 genotype treatments, although the 16 treatment combination still produced significantly less CO_2 than the genotypes in monoculture ($t = -1.76$, $p = 0.015$).

CO_2 efflux at $t = 16$. Treatment identity had a significant influence on respiration (Fig 3A, Table 2), although the range of CO_2 efflux between individual treatments had narrowed to approximately 2–6 mg CO_2 d^{-1} from $t = 10$. The greatest variation at this time point occurred between two genotypes of the same species, treatments A and D of the *A. rubescens* species group ($t = 6.07$, $p < 0.001$). Patterns between individual treatments were similar to those seen at $t = 10$, although many of the original higher CO_2 producing monocultures exhibited deteriorating production rates. Curiously, where treatment AFKP was respiring less than treatment BEJO at $t = 10$, the reverse was occurring at $t = 16$, indicating that time

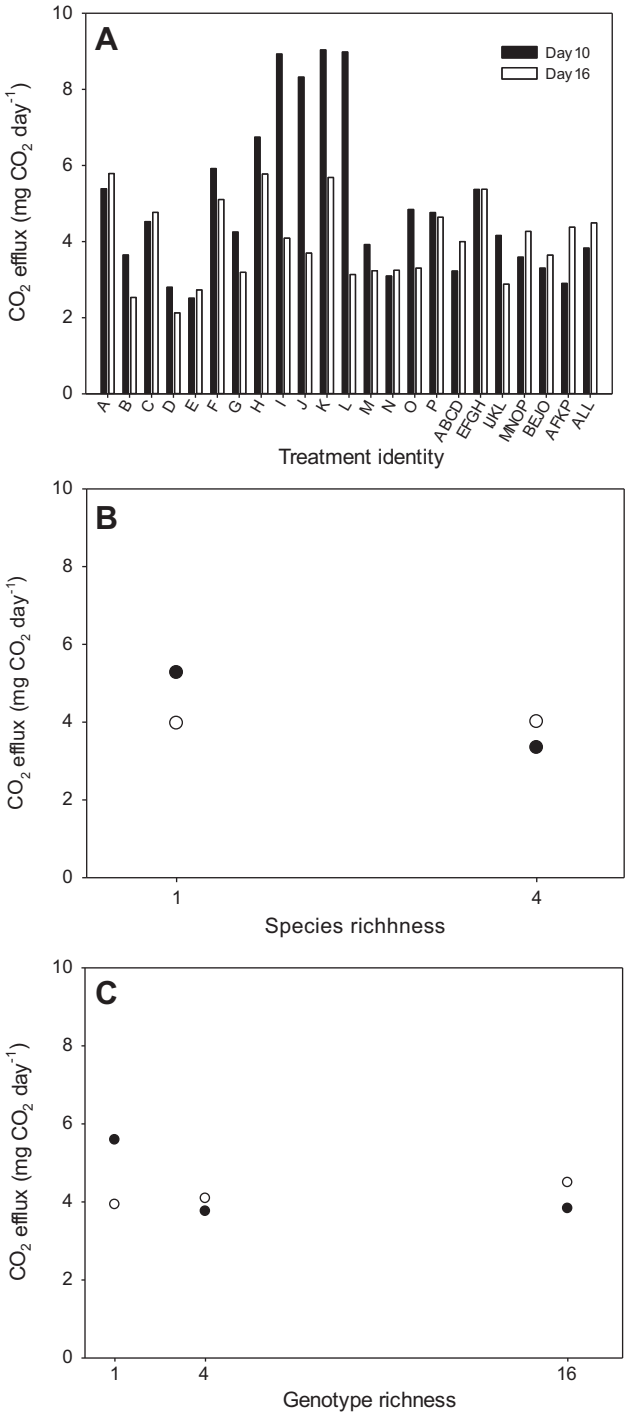

Fig 3 – The effect of (A) treatment identity, (B) species richness and (C) genotype richness on the CO_2 efflux (mg CO_2 d^{-1}) of species and genotypes of EM fungi at 10 d (black bars/circles) and 16 d (white bars/circles). Axis labels as in Fig 1. In (A), treatment identity had a significant influence on fungal respiration on day 10 (L-ratio = 96.55, d.f. = 24, $p < 0.001$) and day 16 (L-ratio = 61.41, d.f. = 24, $p < 0.001$). In (B), species richness lead to a significant decrease in CO_2 efflux (L-ratio = 15.80, d.f. = 3, $p < 0.001$) at 10 d but not at 16 d (L-ratio = 0.32, d.f. = 2, $p < 0.851$). In (C) CO_2 efflux also decreased significantly with genotype richness (L-ratio = 19.75, d.f. = 4, $p < 0.001$) at 10 d but not at 16 d (L-ratio = 0.56, d.f. = 2, $p = 0.851$).

and stage of development between different species and combinations of species is an important factor in CO_2 production. Furthermore, contrary to what was reported at $t = 10$, CO_2 efflux increased slightly with genotypic richness (Fig 4C), although this effect was not significant.

Species richness, genotype richness and treatment identity effects on the C and N parameters of dried fungal material

Regression analyses showed significant positive relationships between % N of the dried fungal material and the biomass of the individual fungal communities ($R_2 = 0.34$, $t = 7.58$, $p < 0.001$) and the CO_2 efflux at $t = 10$ ($R_2 = 0.38$, $t = 8.61$, $p < 0.001$) and $t = 16$ ($R_2 = 0.25$, $t = 6.19$, $p < 0.001$). There were significant but weak relationships between % C of the dried fungal material and the biomass of the fungal communities ($R_2 = 0.04$, $t = 2.09$, $p = 0.039$) and the CO_2 efflux at $t = 10$ ($R_2 = 0.04$, $t = 2.10$, $p = 0.038$). Treatment identity (Fig 4, Table 2) had a significant effect on the % C, % N and the C:N ratio of the dried fungal material of the communities. Percentage C values of genotypes ranged from 40.6 % in treatment E (*P. fallax*) to 44.7 % in treatment A (*A. rubescens*) ($t = 3.32$, $p = 0.001$). However, intraspecific variation of % C was almost as great as interspecific variation; for example, genotypes of *P. fallax* ranged from 40.2 % (treatment E) to 44.2 % C (treatment F; $t = 3.09$, $p = 0.003$). Similar patterns were observed for the % N and C:N ratios of the fungal tissue, with high levels of variation occurring both between and within species. Increased species richness caused a significant decrease in the % N of the dried fungal community material (Fig 5, Table 2). However, no other C/N parameters were affected by changes in richness.

Transgressive overyielding (D_{max})

All treatments underyielded in biomass production, CO_2 efflux and % N compared with their highest performing component species in monoculture. We found that for biomass, there was a marginal propensity for transgressive overyielding (D_{max}) to decrease with intraspecific richness (Table 2, Fig S1). However, CO_2 efflux on both time points and % N were not significantly affected by increasing genotypic richness.

Discussion

This study is the first to test the interactive effects of genotypic and species richness of fungi on productivity, nutrition and activity. Previous manipulations of EM species diversity have reported mixed effects of diversity on host plant responses (Baxter & Dighton 2001, 2005; Jonsson *et al.* 2001), and positive effects on C cycling and productivity within *in vitro* assembled communities of EM fungi, both in terms of species richness (Wilkinson *et al.* 2012) and genotypic richness (Wilkinson *et al.*, 2010) through a mixture of selection and complementarity effects. Our study highlights the significant variation of productivity and nutrient concentrations of EM fungi when grown *in vitro* at the genotype level, and thus the potential importance of genotype and species identity for C cycling. Moreover, it shows that more diverse communities

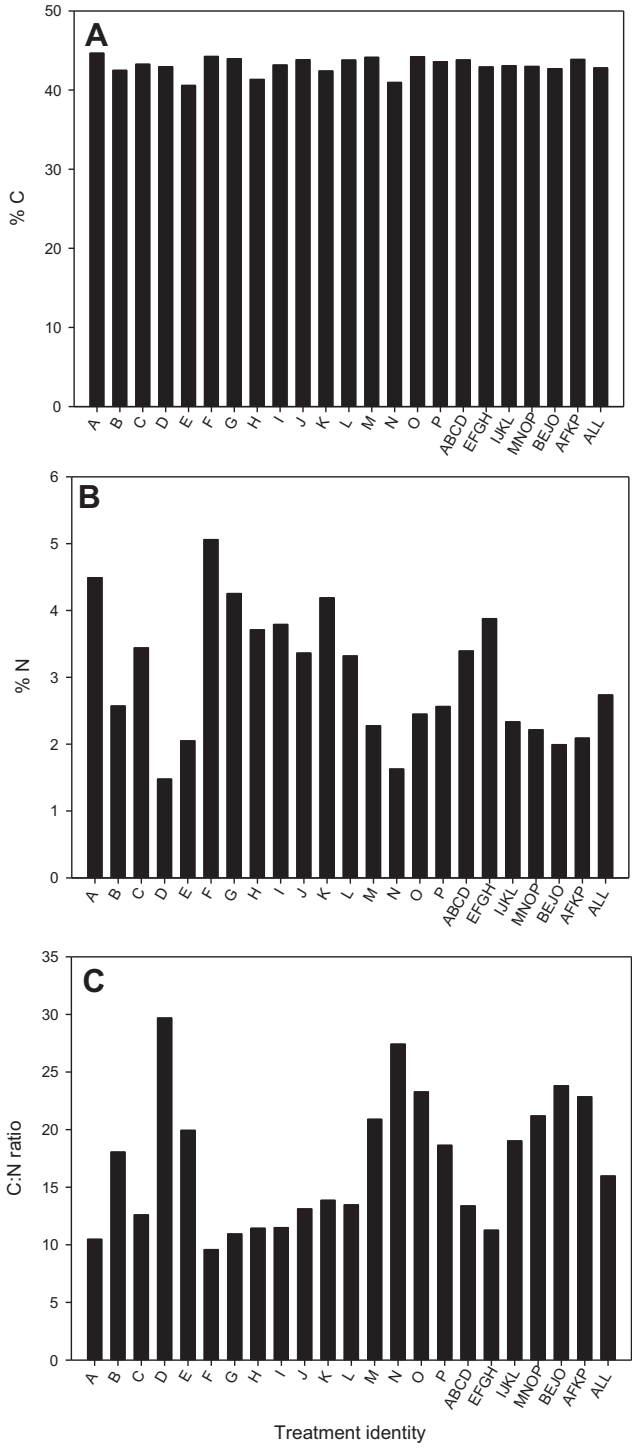

Fig 4 – The effect of treatment identity (A) % C, (B) % N and (C) C:N ratio of the community fungal matter at the end of the study period. Axis labels as in Fig 1. Treatment identity had a significant effects on (A) % C (L-ratio = 70.99, d.f. = 24, $p < 0.001$), (B) % N (L-ratio = 142.31, d.f. = 24, $p < 0.001$) and (C) C:N ratio (L-ratio = 119.35, d.f. = 24, $p < 0.001$).

can behave differently to their component genotypes, leading to overall declines in productivity with increasing genotype and species richness, and thus refuting our hypothesis that increasing diversity would increase productivity.

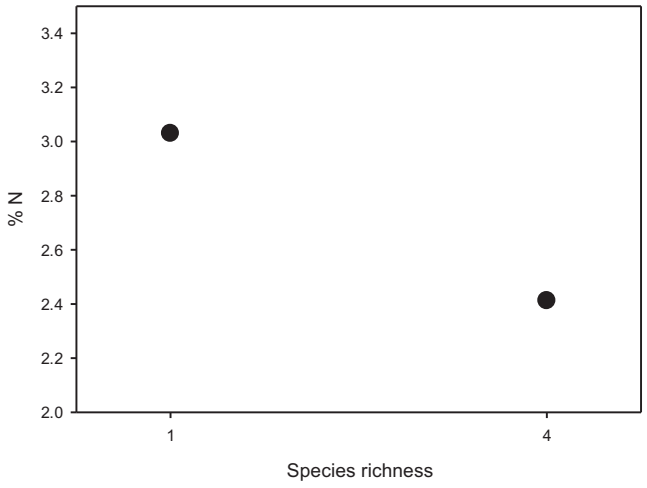

Fig 5 – The effect of species richness on the % N content of the fungal material in the communities. For each graph the circles represent predicted values from the optimal regression model. Species richness had a significant influence on % N (L-ratio = 6.62, d.f. = 3, $p = 0.010$).

Treatment identity effects

The effects of treatment identity on biomass production, CO_2 efflux and fungal C, N and C:N ratio were highly significant, and variation among species in monoculture was comparable to within-species variation for a number of fungi. Genotype identity of fungal endophytes and parasites has previously been found to strongly influence the persistence of symbioses (Rudgers *et al.* 2010), pathogenicity (Daayf & Platt 2003) and host plant–parasite relationships (Bultman *et al.* 2003). When comparing leaf litter decomposition and N release dynamics between genotypes of *S. altissima* and between three different species of *Solidago*, Crutsinger *et al.* (2009) also found that plant species and genotype identity had a much more important effect on leaf litter decomposition and N release dynamics than genotype diversity, although variability always remained much higher between the *Solidago* species than between the genotypes of *S. altissima*. In our study, a striking effect of genotype identity was that in the later stages of EM community development, intraspecific variation (between *A. rubescens* genotypes) in CO_2 efflux was greater than interspecific variation, which highlights the ecological importance of identity at the level of the genotype for populations of soil fungi.

Our study suggests that the N content of fungal material influences CO_2 efflux and biomass production of fungi in pure culture. For example, genotype L, a *Suillus* genotype with a moderately high N content, also produced high amounts of CO_2 during the early stages of the experiment. These findings could have implications for carbohydrate demands placed by different EM fungal genotypes and species on host plants in the field. Indeed, a large proportion of fine root respiration is attributable to root tip N concentrations, which in turn is influenced by the identity of EM associates (Trocha *et al.* 2010). The effects of genotypic identity of EM fungi in association with host plants on productivity and CO_2 efflux thus warrant further investigation.

Genotype and species effects

EM species identity (Jonsson et al. 2001; Wilkinson et al. 2011), and genotypes (Wilkinson et al. 2010) have previously been found to underpin strong effects of EM species/genotype richness on the productivity of both plant and fungal partners. In a striking contrast to what has previously been reported (Wilkinson et al. 2010, 2012) we demonstrate here that increased EM fungal richness lead to a small but significant decrease in CO_2 efflux between the monocultures and mixtures of four and 16 species/genotypes. This study used many genotypes and species whose interactions have not been tested in earlier studies, and the negative effects of richness may have been caused by antagonistic interactions between genotypes of different species. The responses of mixed treatments did not necessarily reflect those seen in monocultures of the comprising species; for example, the *Suillus* mixed genotype community produced less biomass and CO_2 than all of its highly productive components, and treatment AFKP, the community that contained all of the 'best performing' genotypes, produced less biomass and CO_2 than the 'worst performing' genotypes. Given the increased propensity of mixed species/genotype treatments to underyield (i.e. produce less biomass in mixture than the best performing component species in monoculture) in biomass production with increasing diversity, it is possible that there were strong antagonistic complementarity (competition strategies for resources, as defined by Loreau & Hector (2001)) and selection effects (dominance by a less productive genotype) operating within the populations/communities, potentially enhanced by the limited space and resources in the microcosms. Although plant mixtures often perform better than monocultures (e.g. Cardinale et al. 2006, 2007), several studies have demonstrated negative effects. For example, Polley et al. (2003) found that mixtures of the grasses *Gaillardia pulchella*, *Monarda citriodora* and *Lolium perenne* consistently underyielded at high density compared to monocultures, as a result of negative or antagonistic interactions among species and selection effects that favoured *L. perenne*, the least productive species. Hooper (1998) found evidence of competitive suppression by a lesser performing group of species on a highly productive group of species, resulting in a lack of response in productivity to higher diversity treatments.

The N content of the fungi also decreased significantly with increasing species richness which suggests that either N was not being taken up as efficiently in these communities or it was being used for something other than biomass production, such as extracellular enzyme production, in order either to obtain more nutrients or to inhibit the growth of the surrounding fungi. Species of EM fungi are known to produce a variety of extracellular compounds (Leake & Read 1990; Hodge et al. 1996; Tibbett et al. 1999; Leake et al. 2002), and in certain situations recognition of 'non-self' by basidiomycete mycelia is known to trigger the release of volatiles, extracellular enzymes and secondary metabolites, thus leading to changing patterns of resource use and differences in colony morphology and growth rate (Malik & Vilgalys 1999). However, fungal N content was not affected significantly by genotype richness and it is likely that extracellular enzyme production is not induced to the same degree by genotypes of the same species or in the highest diversity community. Here, other forms of negative and/or antagonistic interaction may be occurring that promote the growth of less productive species at the expense of others, and it is possible that in the most diverse treatment (ALL) some species/genotypes became extinct as a result of the high community richness, thus supporting theories that when competition interactions dominate in a system, increasing diversity in one species group may reduce diversity in another as a result of filling the available niches (Vellend 2008).

We found that time had a significant effect on the amount of CO_2 produced between different treatments and levels of richness. At the start of the study CO_2 efflux was greatest overall in the monocultures and it is likely that available resources were rapidly depleted from these treatments. However, towards the end of the study, CO_2 efflux showed signs of increasing alongside diversity, possibly as a result of slower initial resource depletion in mixed treatments through antagonistic complementarity and selection effects. Time can lead to significant changes in community biodiversity effects, yet it has often been overlooked in short-term diversity-manipulation studies (Jonsson 2006; Cardinale et al. 2007). It is possible in our study that biomass and CO_2 production may have begun to increase alongside diversity due to increased complementary resource use under depleting nutrient availability, had measurements continued. In a recent meta-analysis of studies that have tested the effects of diversity on plant productivity, Cardinale et al. (2007) found that studies that had been running for longer periods of time (e.g. multiple generations/growing periods) tended to show more net biodiversity effects and there was increasing evidence of polycultures overyielding compared with monocultures. They concluded that this is because complementarity effects may grow stronger over time (Tilman et al. 2001; Spehn et al. 2005; Van Ruijven & Berendse 2005; Fargione et al. 2007). The 'closed' microcosm system is not strictly representative of true field conditions where there is a constant influx of material from organic matter decomposition and a continuous delivery of sugars from host plants. However, boreal forest soils are typically N-depleted and it is therefore possible that emerging complementarity effects between species over longer time periods could also occur in the field.

Under more complex, organic matter rich and spatially heterogeneous forest soil conditions it is most likely that facilitative interactions and niche differentiation are likely to play considerable roles in EM ecosystem functioning due to inter- and intraspecific differences in the ability to exploit resources spatially, and produce extracellular enzymes. Furthermore, field soil can undergo significant environmental fluctuation and disturbance, and evidence from both plant (Mulder et al. 2001) and fungal (Toljander et al. 2006) diversity studies suggests that niche differentiation and facilitative interactions can play an important role in maintaining ecosystem functioning in diverse communities under variable conditions. However, this study does provide a conservative test for the previously unexplored role of combined genotype and species diversity in belowground ecosystem functioning by removing confounding factors, such as the presence of host plants which have been shown to influence the community structure of their EM partners (Korkama et al. 2006).

Conclusions

Fungal genotype identity had strong effects on EM productivity, although in mixtures of genotypes and species, fungi did not necessarily behave in the same way as they did in monoculture. This indicates that antagonistic complementarity and selection effects play important roles in EM population and community functioning, at least in microcosm conditions. We have also demonstrated that variability in functional traits between fungal genotypes can be as great as that seen between species, which may have implications for the structure and functioning of populations and communities of EM fungi in the field. A recommendation for future research would be to examine if genotypic diversity effects are also seen in more heterogenous, complex substrates with host–plant interactions. Whether competition for resources in multi-genotype and species communities of EM fungi is as intense under higher niche availability remains to be tested.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.funeco.2012.01.005.

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